Distribution Patterns of Immatures of the Diamondback Moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and Its Larval Parasitoid on Cabbages

Annamalai SIVAPRAGASAM, Yosiaki ITÔ and Tetsuo SAITO

*Laboratory of Applied Entomology and Nematology, Faculty of
Agriculture, Nagoya University, Nagoya 464, Japan*

(Received March 24, 1986)

The distribution patterns of the immature stages of the diamondback moth, *Plutella xylostella*, on cabbages were contagious. IWAO's (1968) $m-m$ regression method showed that the basic component of the population was an aggregation of eggs for the egg stage but a single individual for the larval and the pupal stages. The significant change of α between the egg and the larval stage suggested that action of mortality factors, viz, rainfall and natural enemies, and dispersal of newly-hatched larvae might be responsible for the change. On the other hand, the similar values of α and β in larval and pupal stages suggested the absence of density-dependent dispersal or action of effective natural enemies. The application of *Bacillus thuringiensis* did not cause any change in the distribution pattern among the treated plots. The distribution pattern of the parasitoid, *Apanteles plutellae* was contagious.

INTRODUCTION

Spatial distribution pattern is an important ecological property of a species (e.g., SOUTHWOOD, 1978), not only to decide sample size required to get a reasonable level of precision, but also to analyse the population processes such as density dependence of a certain mortality process (IWAO, 1968).

This study elucidated the distribution pattern of the immature stages of *Plutella xylostella* (L.), an important pest of cabbages. To date only a few studies are available, particularly on the distribution pattern of immatures (e.g., HARCOURT, 1960; CHUA and LIM, 1977), and in these studies the aggregative pattern of the insect was described using the parameter k of the negative binomial distribution. In this study, the distribution patterns of *P. xylostella* were examined using the mean crowding-mean ratio which has been found to be more reliable and ecologically meaningful. The specific objectives were to determine the changes in seasonal distribution patterns of the immatures of *P. xylostella* and its major larval parasitoid *Apanteles plutellae* (KURDJ) (Hymenoptera: Braconidae) on cabbage, and determine the effect of the microbial insecticide *Bacillus thuringiensis* on the distribution patterns of immatures.

MATERIALS AND METHODS

Experimental plots and treatments. The experimental plots were located at the Nagoya University Farm at Togo, Aichi Prefecture. There were three trials; the first lasted

from early May to mid-July 1984 (hereafter called summer cabbage), the second from early October 1984 to late March 1985 (hereafter called winter cabbage) and the third, a part of which was treated with *B. thuringiensis* (see below), lasted from early May to the middle of July 1985 (hereafter called *B.t.* cabbage).

The summer and winter cabbage plots were 20 × 10 m. Each plot consisted of 600 cabbage plants in 14 rows with ca. 43 plants per row. The distance between rows was 75 cm and that between plants within a row was 50 cm. No insecticide was used although other cultural practices, e.g. fertilizing, were carried out in accordance with local farmers' practice.

The *B.t.* cabbage plot was 20 × 20 m and consisted of 20 rows of cabbage planted as described above. The treatments consisted of: (1) unsprayed check, (2) a single spray of a non-commercial *B. thuringiensis* (freeze-dried powder of *B. thuringiensis* subspecies *kurstaki* containing spores, Lot No. 12K-1, Shionogi Co. Ltd. Aburahi Laboratories, Shiga) and (3) a single spray of a commercial *B. thuringiensis*, which contains toxin only (Toarow CT®, Toa Gosei Kagaku, Nagoya). The cabbage field was divided into nine plots of 5 × 2.5 m with 40 cabbage plants per plot. Each treatment, replicated three times, was arranged in a randomized-block design. Each plot (replicate) was separated from others by a barrier of four rows of cabbage between treatment columns and two rows between treatment rows. The size of the cultivated barrier was considered sufficient since there is little or no inter-plant dispersal of *P. xylostella* larvae (HARCOURT, 1961), and to avoid insecticidal drift, if any, although spraying was done on an apparently windless day. The *B. thuringiensis* was applied at a concentration of 0.1% aqueous suspension of water (pH ca. 7.25) with 0.0001% Linuh® spreader/sticker (Nihon Nohyaku, Tokyo). Spraying was done at the time of peak larval population in the field.

Sampling unit and census methods. The individual cabbage plant was used as the basic sampling unit ('quadrat') throughout the study.

For population census, both the summer and winter cabbage plots were divided into four quadrats consisting of 150 plants. Direct countings of eggs, larvae 1 (1st and 2nd instars), larvae 2 (3rd and 4th instars) and pupae (including prepupae) were made twice weekly at 3 or 4 day intervals on 10 to 15 randomly sampled plants per quadrat. For winter cabbage, from January onwards, sampling was done once per week due to the low population in the field. For *B.t.* cabbage, a total of 30 plants (10 plants per replicate) per treatment were sampled as above, from 12 days after the transplantation of cabbage until the end of the crop. To estimate the number of the parasitoid, *A. pluteolae*, the number of cocoons on each plant was counted during the census of the immature stages. As far as possible, places of all cocoons on cabbage leaves were marked with a felt pen to avoid recounting on the following census days.

Analytical methods. For distribution analysis, the two larval categories (larvae 1 and larvae 2) were pooled to overcome the problem of low larval density during winter and on *B.t.* cabbage. There was no significant difference in the values of Iwao's $\hat{m}$ - m regression parameters between larva 1 and larva 2 on summer cabbage.

The changes in spatial distribution patterns with time were studied using the LLOYD (1967) 'patchiness' index, $\hat{m}/m$, which is greater than unity for contagious, equal to unity for random and smaller than unity for uniform distributions. Here,

$$\hat{m} = \frac{1}{N} \sum_{i=1}^n x_i(x_i - 1) \quad (1)$$

where x_i is the number of individuals in the i th quadrat, n is the number of quadrats (plants) and N is the total number of individuals.

The regression of mean crowding ($\bar{m}^*$) on the mean density (m) (Iwao, 1968) was used to describe the overall trend in distribution. Iwao (1968) reported that for various distribution models $\bar{m}^*$ varies linearly with m , that is:

$$\bar{m}^* = \alpha + \beta m \quad (2)$$

where α and β are inherent characteristics for a particular distribution model. The intercept α , termed the 'index of basic contagion' indicates whether the basic component of the population is a single individual ($\alpha=0$) or a group of individuals ($\alpha>0$). The regression coefficient β , termed the 'density-contagiousness coefficient', describes the manner in which the basic components distribute themselves in their habitat with changing mean density.

RESULTS

Temporal variations in distribution pattern

Figure 1a, b (bottom) shows temporal trends of the number of insects and distribution index $\bar{m}^*/m$ of immature population. The $\bar{m}^*/m$ values showed that the distribution pattern of immature stages of *P. xylostella* was contagious throughout the crop in both the summer cabbage and control plot of *B.t.* cabbage, despite the wide fluctuations in population density (Fig. 1a, b, top). It was also observed in summer cabbage that the distribution of eggs was generally most contagious, while that of pupae was near random; however, this trend was not observed in *B.t.* cabbage. The values of $\bar{m}^*/m$ were not calculated for winter cabbage due to rather low population density and small sample size (Fig. 2).

Relationship between $\bar{m}^$ and m*

The relationship between $\bar{m}^*$ and m for the various immature stages fitted well

Fig. 1. a, b: temporal trends in the number of insects per plant (top) and $\bar{m}^*/m$ (bottom). Symbols: ($\blacktriangledown$) egg, (∇) larva and ($\blacksquare$) pupa.

Fig. 2. Population trends in winter cabbage. Symbols: (▼) egg, (▽) larva and (■) pupa.

Fig. 3. a-d: relationship between $\bar{m}^*$ and m for all trials for (a) egg, (b) larva, (c) pupa and (d) the parasitoid, *Apanteles plutellae*. Symbols: (○) summer cabbage, (●) control plot of *B.t.* cabbage, and (▼) winter cabbage. -----: random line ($\alpha=0$, $\beta=1$).

($P<0.001$) to straight lines (Fig. 3a-c). The values of α suggested that the basic component of distribution was an aggregation of eggs ($\alpha>0$, see Fig. 3a) but a single individual ($\alpha=0$, Fig. 3b, c) for both the larval and pupal stages. Further, α decreased from the egg to the larval and pupal stages ($P<0.001$, F test). However, there was no significant difference in α between the latter two stages. The values of β were always greater than unity and there was no significant difference in β values among the three developmental stages.

Distribution patterns in *B.t.* plots

For each developmental stage, there was no significant difference in either α or β of the $\bar{m}^*$ - m regression lines among the two *B. thuringiensis* treatments and control plots in *B.t.* cabbage (Table 1). Therefore, data were pooled for a common regression analy-

Table 1. The estimated linear regression parameters of mean crowding $\bar{m}$ on mean density m for two *B. thuringiensis*-treated (non-commercial and Toarow CT) and control cabbage plots*

Stage	Control			Non-commercial			Toarow CT		
	α	β	r^2	α	β	r^2	α	β	r^2
Egg	6.23	1.09	0.98	6.96	1.12	0.88	7.78	1.07	0.96
Larva	1.16	1.12	0.98	0.72	1.05	0.97	1.06	1.12	0.95
Pupa	0.29	1.13	0.94	0.07	1.32	0.88	0.51	1.19	0.80

* No significant difference ($P < 0.05$) in intercept (α) and slope (β) was observed between treatments for each stage.

Fig. 4 a-c: relationship between $\bar{m}$ and m for *B.t.* trial. (a) egg, (b) larva and (c) pupa. Symbols: (●) control, (◐) non-commercial *B.t.*, and (○) Toarow CT. -----: random line ($\alpha = 0$, $\beta = 1$).

sis. Figure 4a-c shows that data from the three different treatments fitted well ($P < 0.001$) to a single regression line, indicating that there was no fundamental change in the distribution pattern by *B.t.* treatments within the observed ranges of mean densities. Values of α for the egg stage were significantly larger ($P < 0.001$) than those of larva and pupa, but β did not show any significant change among developmental stages.

Distribution pattern of a parasitoid *A. plutellae*

Figure 3d shows the $\bar{m}$ - m regression analysis for the number of cocoons of the parasitoid *A. plutellae*, using pooled data from summer cabbage and the control plot of *B.t.* cabbage. There was no *A. plutellae* on winter cabbage. Although r^2 was small (0.44) (Table 1), Fig. 3d suggests that the distribution pattern of *A. plutellae* might be contagious because most of the points were above the random line. It may be relevant to note that the estimate of the parasitoid density in the field based on cocoons might be low. In the laboratory, as high as 57 percent parasitism by this parasitoid was obtained from field-collected larvae. Consequently, there might be some mortality of parasitized larvae due to other undetected factors in the field.

DISCUSSION

The overdispersed or contagious distribution pattern is a common feature in many insect populations. The distributions of the immature stages of *P. xylostella* were, as indicated by $\hat{m}/m$, contagious on most sampling dates.

The values of α and β from pooled data for all trials suggested that the basic distributional unit of *P. xylostella* may be an aggregation of eggs for the egg stage and an individual for larval and pupal stages. IWA0 (1970) suggested that the spatial distribution of eggs of an animal is closely related with the behaviour of gravid females, such as their mobility, preference for oviposition sites and egg laying habit. For *P. xylostella*, an obvious factor causing the contagious distribution of eggs is its egg-laying habit. In this study, a maximum of 74 eggs per plant was recorded on cabbage. With respect to preference for oviposition site, WAN (1970) reported that the adult *P. xylostella* failed to oviposit in unfavorable areas such as those damaged by other insects. In addition, there may also be other undetected differences in host plant quality and microclimate which may cause the contagiousness in egg oviposition by *P. xylostella*. The decrease in α from the egg to the larval stage may indicate the typical pattern of reduction in egg numbers per plant both by action of mortality factors and dispersal of early larvae. As for mortality factors, rainfall, which is a density independent factor and abundant in Japan during early summer, may be important. As suggested by IWA0 (1968), the operation of a density independent factor could alter α . A number of natural enemies, e.g., *Trichogramma* sp, have been recorded during the egg stage (IGA, 1985). Although not determined in this study, HARCOURT (1961) suggested dispersal of newly-hatched larvae from crowded to less crowded cabbage plants.

One of the factors causing the contagious distribution of *P. xylostella* larvae may be the contagious distribution of eggs. Further, there is little or no inter-plant dispersal of larvae (HARCOURT, 1961). The non-significant change of α and β between larvae and pupae suggested the little or no density dependent processes of dispersal (but see HARCOURT, 1961) and ineffective operation of mortality factors during this time interval.

As indicated by $\hat{m}/m$ of summer cabbage, the distribution of eggs was generally most contagious while that of pupae was near random; however, this was not observed in *B.t.* cabbage. HARCOURT (1960) also observed that the degree of contagiousness in *P. xylostella*, based on the index k of the negative binomial distribution, decreases from the egg to the pupal stage. In this study, the decrease in the degree of contagiousness from the egg stage, as seen in summer cabbage, might be due to the decrease in α , since β values were not significantly different among the stages.

The similarity in the degree of contagiousness in control and *B. thuringiensis* treated plots suggested that it might not be necessary to change the sampling strategy (e.g., determination of sample size) for *P. xylostella* as a result of application of *B. thuringiensis*.

HASELL (1978) suggested that the contagious distribution of a parasitoid might have an important implication on its efficiency as a biological control agent. But, parasitoid aggregation (based on contagiousness) need not necessarily lead to spatially direct density-dependent mortality (MORRISON and LEWIS, 1984); in fact, studies on life tables (SIVAPRAGASAM, 1986) suggested that the relationship between *A. plutellae* and its host (larvae) might be delayed-density dependent. Further, the ephemeral nature of the cabbage ecosystem may also affect the efficiency of *A. plutellae* against *P. xylostella*.

The contagious distribution of *P. xylostella* has an important influence on the sampling procedures. That is, the minimum sample size to be taken with a given degree of precision must be rather large, particularly at low population densities. Sample size estimates for the larval stage, using the equation provided by IWAO and KUNO (1971) with $\alpha=0.68$, $\beta=1.34$ (for pooled data, see Fig. 3b), $D=0.2$ and $t_{0.05}=2$ were 40–70 plants when the mean larval density of *P. xylostella* was 5–20 per plant, and more than 90 when the density was lower than 0.5 per plant. However, if we neglect t^2 in the sample size equation as many field entomologists do (e.g. SOUTHWOOD, 1978), the sizes are 11–17 and 90, respectively; reasonable values for actual forecasting work.

ACKNOWLEDGEMENTS

We gratefully acknowledge the valuable suggestions given by Drs. Yoshitaka TSUBAKI and Kohji HIRANO of our laboratory. We are also very grateful to Dr. Shigekata YOSHIDA, University Farm, Nagoya University, for preparing the cabbage fields, and Mr. Teruo NAKAGOME of the Horticultural Research Division, Aichi-Ken Agricultural Research Centre for supplying the cabbage seedlings. The non-commercial *B. thuringensis* material was kindly provided by the Shionogi Co. Ltd., Aburahi Laboratories, Shiga. Comments by an anonymous referee on the manuscript were helpful.

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